Arkansas
Agricultural Experiment Station
Fayetteville, Ark.

BULLETIN No. 105.
(TECHNICAL)

STUDIES ON THE BACTERIOLOGY OF INFECTIOUS SWINE DISEASES.

R. R. DINWIDDIE,
J. F. STANFORD.

The Bulletins of the Station are Sent Free to All Residents of the State who Request them.

1910.

Agricultural Experiment Station.

BOARD OF TRUSTEES.

His Excellency, GEORGE W. DONAGHEY, *Chairman.*

HON. MARCELLUS L. DAVIS.
HON. W. L. GOODWIN,
HON. GUSTAVE JONES.
HON. GEO. B. COOK.
HON. F. P. HALL.
HON. JOHN F. RUTHERFORD.
HON. R. O. HERBERT.
HON. G. T. BRECKINRIDGE.

HON. JOHN NEELLY, *Secretary.*

BOARD OF CONTROL.

Committee on Agricultural Department, President of the University, Director of the Station.

STATION STAFF.

C. F. ADAMS .... DIRECTOR AND ENTOMOLOGIST
R. R. DINWIDDIE .... *Consulting Pathologist and **Bacteriologist***
ERNEST WALKER .... Horticulturist
V. A. HOOPER .... Dairy Husbandman
W. LENTON .... **Veterinarian**
W. M. BRUCE .... **Chemist**
MARTIN NELSON .... Agronomist
J. LEE HEWITT .... **Plant Pathologist**
A. K. SHORT .... Animal Husbandman
W. S. JACOBS .... Assistant Agronomist
C. H. TOURGEE .... Assistant Dairy Husbandman
J. F. STANFORD .... **Assistant Veterinarian**
H. D. YOUNG .... **Assistant Chemist**
PAUL HAYHURST .... **Assistant Entomologist**
A. P. BOLES .... Assistant Plant Pathologist
B. M. GOW .... **Assistant Veterinarian**
M. B. PATS .... Assistant Animal Husbandman
J. R. TUCKER .... Assistant Chemist
C. V. RUZEK .... Assistant Agronomist
L. L. WOOTTON .... **Executive Clerk**

Correspondents should notify the Director of any change of Post Office Address, error in Address, or failure or delay in receiving Bulletins. When a change of address is desired, both the old and the new address should be given.

C. F. ADAMS, Director,
Fayetteville, Ark.

STUDIES ON THE BACTERIOLOGY OF INFECTIOUS SWINE DISEASES.

R. R. DINWIDDIE.
J. F. STANFORD.

Our knowledge of the etiology of the prevalent porcine plagues of this country has been obtained almost entirely by the investigations of the Department of Agriculture, beginning with the publications of Salmon and Smith on "Hog Cholera" (1889) and of Theobald Smith on "Swine Plague" (1891) and continued later by their successors in this line of work. The latest contributions from this source, those of Dorset, McBryde and Niles (1905 and 1908), now well known to students of comparative pathology, and (by dint of extensive advertising) to the reading portion of the agricultural population generally, reopen the question as to the actual relation of Theobald Smith's hog cholera and swine plague bacilli to these diseases. The *duality* of these prevalent swine epidemics, which, following Smith's differentiation into swine plague and hog cholera, has been generally accepted by veterinary pathologists, would seem also to require further study, since swine plague bacteria are just as frequently associated with these outbreaks of disease which go by the name of hog cholera as are the hog cholera bacilli. In our experience, as will be noted, they are even more frequently encountered. If the *Bacillus cholerae suis* invades the system of the animal secondary to the infection with the primary or essential filterable virus of Dorset it is certain that, in some outbreaks at least, the invasion of the swine plague bacteria is also secondary. Whether or not this is constantly the case when the swine plague bacteria become generalized throughout the animal, can only be learned by further investigation in the light of these recent discoveries. In our own investigations swine plague bacteria have been most frequently found in localized lesions (pneumonic) when the disease showed but slight infectious features and generalized in some highly infectious and disastrous outbreaks.

Besides these reports of the Bureau of Animal Industry, dealing with hog diseases as encountered in the east and west central State

but little additional bacteriological studies of outbreaks of infectious swine diseases have been made (or published) in this country. The existence especially of Smith's swine plague as a distinct and separate infectious disease of American hogs seems to rest on the results of his researches alone. There is evidently need of further bacteriological study of infectious hog diseases as they occur in all sections of the country before any verdict can be given as to the unity or duality of hog cholera.

In our experience in the investigation of outbreaks of hog diseases in this State we have several times been confronted by the conflict of anatomical lesions and bacteriological findings. Ulcers of cecum and colon and splenic engorgement are regarded as the predominant anatomic features of cholera; pneumonia, fibrinous pleurisy and pericarditis those of swine plague. In hogs that present the former postmortem lesions, in cases not too chronic, the *Bacillus cholerae suis* occurs with great frequency disseminated throughout the abdominal viscera. So far as I am aware it has never been found in virulent form except in association with this disease, hence has been regarded as pathognostic of it. Virulent swine plague bactèria may with equal uniformity be isolated from typical pneumonic lesions or may in such cases be obtained from any part of the system. These are the usual bacteriological findings which have led to the belief that these organisms are causative in their relation to the anatomical lesions even if secondary to the actual infection of the animal.

Evidence on these points must be sought by bacteriological investigations of outbreaks of the naturally acquired disease in diverse localities, and by direct experimentation. The present contribution gives the results of the writers' observations under somewhat limited opportunities in a country where hog-raising is necessarily a somewhat restricted industry and pathologic material seldom conveniently available.

OUTBREAK I. CONJOINED CHOLERA AND SWINE PLAGUE.

The disease broke out on a nearby farm and, after the most of his hogs had died, the owner brought in two sick shoats to the laboratory for examination. These were placed for observation in a pen previously unoccupied. Both of these pigs died within a week and showed on dissection typical ulcerative lesions of cecum and colon, extensive pneumonia involving both lungs, pleurisy and pericarditis with com-

plete fibrinous exudate covering the serous surfaces. By inoculation methods both hog cholera and swine plague bacteria were obtained from each of these animals, the former organism from the spleen, the latter from the lung.

Two pigs which contracted the disease by exposure to these also died with similar lesions. Although the pneumonic changes were less marked it is significant to note that the conjoined disease was reproduced by the exposure. In these animals the swine plague bacterium was apparently not generalized but localized in the thoracic organs.

OUTBREAK 2. ANATOMIC LESIONS OF HOG CHOLERA AND SWINE PLAGUE; BACTERIA OF SWINE PLAGUE ONLY.

The study of this outbreak proved interesting as well as perplexing on account of the disparity between the anatomical and bacteriological findings. It occurred on a farm located in the river valley some ten miles from the laboratory. Along the river hogs had been dying during the preceding spring and winter and it is presumed that infection occurred in this way. During the month that the disease had been prevailing some fifty head had died out of about a hundred in the herd and twenty of all ages were more or less sick at the time. There were no special ante-mortem symptoms other than those usually seen in hog cholera.

Dissections of three animals were made on the farm at this time and the viscera conveyed to the laboratory for bacteriological study. Specimens from two others were brought to the laboratory some days later and also subjected to bacteriological investigation.

Prominent features in the post-mortem findings were engorged spleen and lymph nodes, masses of confluent or isolated ulcers of cecum and colon with black pigmentation of mucosa and in one case two large gastric ulcers with enormously thickened base.* Pneumonic lesions were equally prominent in all cases, the lungs throughout their lower halves being completely solidified with purulent points thickly studded over the cut surface. Pleurisy with adhesions was present in two cases. These lesions give the picture of the conjoined diseases, cholera and swine plague.

*In histologic structure these ulcers differed from the chronic intestinal ulcers of cholera only in the greater development of productive inflammatory tissue.

Bacteriology.—The results of the bacteriological tests in these cases gave a finding of disseminated swine plague bacteria, with absence of hog cholera bacilli. Inoculations from spleen, lung, and cecal ulcers to guinea pigs and rabbits resulted in each case in death from swine plague bacteremia. Deaths occurred in from two to three days. Direct cultures from spleen of hogs failed to reveal hog cholera bacilli but gave free growths of swine plague germs. The agglutinating reaction with *Bacillus cholerae* failed in each case.

One sick pig about six weeks old, the dam of which had died from this disease, survived for twelve days after being brought to the laboratory pens. Dissection showed pneumonia only without intestinal changes, the anterior and middle lobes of right lung being in a condition of red hepatization. Guinea pigs were inoculated from the lung and spleen. The former died in three days of swine plague infection while the latter survived. Direct culture tests also showed a localization in this case of swine plague bacteria in the lung only.

From several other hogs which died during this outbreak blood collections were made and tested with cultures of *Bacillus cholerae* for the agglutinating reaction, and in all cases with negative results. This, however, as will be shown later, is of little significance in diagnosis.

In this outbreak of anatomical hog cholera we failed to find the hog cholera bacillus but, it should be admitted, also failed to prove its absence, for several possible explanations of this result might be advanced: (1) The inoculation tests were invalid because of the more rapidly fatal effect of the swine plague bacteria (hog cholera bacilli seldom proving fatal to guinea pigs before the fourth day). (2) *Bacillus cholerae* may have been present at an earlier stage and later disappeared to be followed by swine plague infection as a sequel. (3) Dissection and bacteriological study of a larger number of animals in this outbreak might have led to the discovery of *Bacillus cholerae* in some, which would entail its possible presence in all.

Some additional data bearing on the same subject were obtained shortly afterwards. Hogs were reported to be dying of some disease on the river farms twelve or more miles further east, many of the farmers having lost nearly all their stock. In this locality several post-mortem examinations were made and material obtained for laboratory study. The result of these studies was similar to that above given—virulent swine plague germs alone found. The patho-

logical changes found on dissection may be exemplified by the following cases: The carcasses of two dead hogs were found lying together on the river bank. Of these, one which was not yet decomposed was examined. Abdomen contained about a quart of reddish yellow fluid. Intestinal worms (*ascarides* and *echinorhynchus*) numerous, some of the former in the bile duct. Spleen and liver show little change, kidney shows hemorrhages beneath capsule, glands of mesocolon discolored; in cecum and colon many ulcers from size of dollar down. They are mostly circular or oval, slightly elevated around periphery and surface black pigmented. This discoloration is general over the uninvolved parts of the mucous membrane. Lower part of colon shows smaller ulcers and also a stretch of about three inches covered by a continuous diphtheritic layer. In the small intestine are several groups of small *echinorhynchus* ulcers. In the pyloric part of stomach a number of ulcers of the size of a dime or less. The lungs show a few small hepatized areas.

Bacteriology.—Rabbit 49 inoculated from hepatized lung was killed on the third day when near to death. It showed a large local hemorrhagic exudate in thigh, congestion of abdominal viscera, thoracic viscera normal. Cover glass smears showed swine plague bacteria numerously in heart blood, spleen and liver. Pure cultures of the same organism were obtained from spleen and liver. Agglutination test with *Bacillus cholerae* negative. Rabbit 48 inoculated from spleen and liver of this hog died in fifty-two hours also with swine plague bacteremia.

From the same neighborhood a sick pig, the remnant of a herd of fourteen, was transferred to the laboratory pen. It died four days later. Sections revealed changes similar to the above in the lungs, a few small, thick-based pigmented ulcers in colon and general pigmentation of mucous membrane. The spleen presented an unusual change; it was much enlarged and throughout hard and carnified and adherent to surrounding viscera (splenitis and perisplenitis).

Bacteriology.—Rabbit 51, inoculated with scraping from ulcer of gut, died in eighty-four hours and yielded a bacteriological diagnosis of swine plague alone.

Guinea pig 54 inoculated with scraping of spleen substance died in four days and nineteen hours, and also yielded a bacteriological finding of swine plague alone. In this case the inoculated animal survived long enough to allow the development and generalization of

cholera bacilli if these were present in the inoculating material. That they were not present was shown by the failure of gas formation in sugar fermentation tubes seeded from the spleen and liver and by the negative result of the agglutination test.* From this hog pure cultures of swine plague bacteria were also obtained directly.

It may be noted also, although the fact has no great significance, that all samples of blood of which a considerable number were obtained from sick or dead hogs in the locality from which these two were obtained gave on examination a negative result to the agglutination test with cultures of *Bacillus cholerae.*

As showing the negative result of bacteriologic tests which may also be met with in some cases, a six-week-old pig (the sole survivor of a small herd), the carcass of which was brought to the laboratory from another farm in this locality, showed, on section, purple discoloration of abdominal cutis, congestion of liver and spleen, pin point extravasates on surface of kidney, less marked on cut surface, congestion of both lungs with pneumonic consolidation of small central lobes, and sclerosis with hard necrotic foci in testes. The large gut was not evidently abnormal but on fundus of stomach was a superficial croupous-like yellow exudate large as a half dollar. The colic lymph nodes were engorged.

Both culture and inoculation tests in this instance failed. Guinea pigs inoculated from stomach lesions, lung and lymph nodes, all remained unaffected.

The various cases here described are sufficient to show that *Bacillus cholerae suis* may be either absent or impossible of demonstration in outbreaks presenting the anatomical lesions of cholera. If an opinion were based on these cases alone one might even infer that the lesions found were the result of the action of swine plague bacteria alone. Theobald Smith in contrasting the necrotic action of swine plague bacteria with those of the hog cholera bacillus on the mucous surfaces ascribed to the former a more superficial action, the formation of a scaly or bran-like exudate on the summits of the intestinal villi, while the latter tend more to deep-seated focal lesions ending in the "button-like" ulcers considered by pathologists as pathognostic of hog cholera. While we have no data to show that such button ulcers may be produced by artificial infection with swine plague bacteria our own experiments, later detailed, will show that infection with

*Rabbits and guinea pigs inoculated with *Bacillus cholerae suis* nearly always give a plain agglutination reaction (miscroscopical) within five days.

Bacillus cholerae by ingestion of cultures frequently occasions a continuous superficial necrosis of the intestinal mucosa with fatal ending prior to the formation of deeper-seated sloughs or ulcers.

Illustrating this similarity between the lesions associated with *Bacillus cholerae* and those ascribed to swine plague germs are the two cases examined in the outbreak next referred to.

OUTBREAK 3. HOG CHOLERA WITH A BACTERIOLOGICAL FINDING OF BACILLUS CHOLERAE ALONE.

The organs of two hogs which had been killed for investigation when in a moribund condition were brought to the laboratory from St. Paul, a small town further up the river than the cases previously described. The few hogs kept there had already mostly died from some disease supposed to be cholera.

Dissection.—Pig 4.12.01. Pieces of lung brought were discolored but spongy and not pneumonic; hemorrhagic spots were visible on surface; spleen slightly enlarged but not appreciably softened or discolored; colic lymph nodes swollen and slightly darkened; cecum covered by yellow exudate not quite continuous, no ulcers; intervening portion of mucosa not pigmented or discolored; colon generally dark red except when covered by this exudate. The exudate is here in patches on elevated "buttons" when rubbed off surface is smooth, white or blood injected—no distinct ulceration. This is the condition throughout colon.

Bacteriology.—Culture from spleen is bacillus cholerae in purity.

Guinea pig inoculated with mixed material from spleen, colic lymph nodes and scraping from mucous surface of colon died in five days with dissemination of hog cholera bacilli throughout the body.

Pig 4.12.01a. *Section.*—Three pieces of lung of which two were discolored but spongy, the third being consolidated; spleen slightly enlarged, dark, and soft; liver shows numerous pin-head and larger sized white patches on surface and section surface; punctiform hemorrhages on kidney; colic lymph nodes enlarged and very dark; colon shows a continuous yellow adherent pseudo-membrane throughout; cecum not on hand; some reddening on serous surface of gut.

Bacteriology.—Culture from spleen gave a mixed growth of *Bacillus cholerae* and *Bacillus coli.*

Guinea pig 91 inoculated from spleen, colic lymph node and exudate of mucous surface of colon dies in three days with purulent peritonitis as the most prominent changes; liver engorged and friable; spleen little changed; lung edematous. This looked like swine plague infection but cultures obtained (liver and spleen) proved to be *Bacillus cholerae*. Guinea pig 92 inoculated from pneumonic patch of lung remained unaffected.

So far as the patho-anatomical changes are concerned the disease in these hogs might have been diagnosed with equal reason either as hog cholera or swine plague.

OUTBREAK 4. SWINE PLAGUE PNEUMONIA IN PIGS.

The two cases here described were sent in from a hog breeding farm with the information that a number of pigs had lately died with the same symptoms.

Pig 5.1.01 about one month old, fat, lies on side and shows jerking respirations or "thumps." Pig died on day of arrival at laboratory.

Section.—Liver pale and mottled (fatty); spleen and kidneys unchanged. Lungs both solid through most of extent, in some parts red and in other grey hepatized; small intestines normal with absence of ascarides; large gut covered by pin-head yellow nodules projecting on serosa except on cecum and first part of colon. Mucous surface shows in cecum an almost continuous yellow adherent scurf; further back in colon this is in the form of separate islets slightly elevated. The nodules before mentioned project on mucous surface also and feel like small shot; they are white and on summit of some a minute dark or yellow spot is seen.

Bacteriology.—Rabbit 69 inoculated from lung lesion died in three days with marked peritonitis and swine plague bacteremia. Rabbit 62, previously vaccinated against swine plague bacteria, inoculated subcutaneously with scraping from mucosa of cecum and colon of pig 5.1.01 died in two weeks in an emaciated condition with an extensive purulent infiltration in the subcuta near site of inoculation. From the spleen was obtained a culture of swine plague bacteria mixed with *Bacterium coli*. Hog cholera bacilli were not found, and agglutination test of blood with *Bacillus cholerae* negative.

Pig 5.6.01, another from the same litter, showed on dissection no intestinal changes; the spleen was enlarged and dark; the right lung had an area about one-third of posterior lobe which was grey hepatized and adherent to ribs, and on other parts some dark red areas from one to two centimeters wide which extended wedge-shape in the substance of the lung.

Direct culture tests gave mixed growths (due to decomposition) from which nothing could be learned. A rabbit inoculated from the lung died in seven days with severe peritonitis and generalized swine plague infection.

This outbreak appeared to be one of pure or unmixed swine plague infection with lesions involving either the thoracic organs alone or these and intestinal tract as well.

From the number of animals (suckling pigs) which died, contagion probably constituted more of a factor in the spread of the disease than in some other instances which we have met with where pneumonia more or less limited in area although associated with the swine plague bacteria has proved almost sporadic in its occurrence. In this respect swine plague pneumonia in pigs is entirely similar in etiology to *Diplococcus pneumoniae* of man where the influence of contagion is also extremely variable.

In another instance, the only case of the kind we have observed, and which may fittingly be described here, the element of contagion was apparently lacking in a case of the conjoined disease—cholera and swine plague.

In a small herd of a half dozen hogs kept in a small field near town one animal died after about two weeks noticeable illness. The symptoms given by the owner were "coughing and thumping." Specimens brought to the laboratory were examined and subjected to bacteriological study.

Section.—Right lung throughout contains numerous white nodes from size of a pea to that of a walnut, hard or with soft core; intermediate parts hepatized; upper part of left lung in similar condition but nodes less numerous and lung more spongy; pleural surface roughened but not adherent. One kidney on section is lighter in color than the other and the pelvic mucous lining is thickened and coated with a yellow slime. Intestines, both large and small, showed no abnormality except that the first part of latter was deeply bile-stained.

Culture Tests from liver and spleen growths were obtained which when subjected to all biological tests proved to be pure cultures of *Bacillus cholerae suis.*

Inoculations.—Guinea pig 106 inoculated from liver and spleen of hog died in nine days yielding pure cultures of *Bacillus cholerae.* Guinea pig 107 inoculated from lung lesion died in three days, yielding pure cultures of swine plague bacteria. The second transfer of this culture, subcutaneously inoculated to a rabbit, killed it in three days of swine plague bacteremia with intense peritonitis.

The blood of this hog possessed marked agglutinating properties for stock cultures of *Bacillus cholerae* as well as for the cultures obtained from its own body.

Although freely exposed none of the other hogs in this bunch ever showed any sickness.

The notable features in this case are (1) the absence of obvious hog cholera lesions; (2) the apparent lack of contagiousness. The virulence of the swine plague germ was of average degree for small animals. The variety of *Bacillus cholerae* obtained, although on first inoculations apparently of rather feeble virulence, proved on later tests to be quite up to the average in this respect. The second transfer from guinea pig 108 when inoculated subcutaneously in dose of one-tenth cubic centimeter to rabbit 94, weighing 1453 grams, caused death in three days with the usual pathologic changes. Its virulence was also shown by feeding tests on pigs carried on some time later.

The thought now naturally arises that this might be a case of pure bacterial infection without the agency of the filtrable virus. It is at least entitled to be called a sporadic case of swine plague, and, if the presence of *Bacillus cholerae* is sufficient for diagnosis, one of sporadic hog cholera as well.

That swine plague, or at least swine plague pneumonia, occurs as a sporadic disease may be illustrated by the following example, one of many similar ones which we have met with:

Pig 9.26.04, one of a lot of four which were confined in the same pen and undergoing some feeding experiments with cotton-seed oil, died after a period of unthrift of several weeks. Dissection revealed advanced pneumonia with suppuration in areas from size of a pin head to that of a pea; pleurisy with adhesions; absence of other obvious changes. Preparations from lung were examined for tubercle bacilli with negative finding (the condition being not unlike advanced

pulmonary tuberculosis of hogs). Cover glass preparations showed several forms, but chiefly in large number vacuole staining ovals, apparently swine plague bacteria. Inoculation tests proved fatal to guinea pigs in twenty-four to forty-eight hours, with generalization of swine plague bacteria.

The virulence of this culture was shown by later tests, one-tenth cubic centimeter proving fatal to adult guinea pigs in from three to five days.

There was no element of contagion manifested in this case, which proved to be solitary.

OUTBREAK 5. VIRULENT DISEASE IN ADULT HOGS ASSOCIATED WITH SWINE PLAGUE BACTERIA.

This occurred in the winter 1904-1905, in a herd of breeding sows which had been returned from exhibition at St. Louis on October 20th, and since their return had, according to the statement of their owner, not been exposed to any known source of infection. First symptoms (scouring) appeared December 6th, and first death December 16th. By January 18th, only one out of the original thirteen remained alive. Five of these animals were dissected and examined bacteriologically so far as their condition allowed. In the others post-mortem change was too advanced to allow of any profitable examination. The result, therefore, is less significant than it otherwise might have been.

From records here given of post-mortem dissections and bacteriological study it will be seen that the conditions on which a diagnosis of cholera are usually based were absent. The anatomical lesions, so far as these are diagnostic in this disease, were referable rather to swine plague. The bacteriology also, where positive findings were obtained, was that of swine plague.

Hog 12.16.04, weight 350 pounds, in show condition, was opened eighteen hours post-mortem, and some decomposition was present. Spleen little enlarged, but dark and soft; part of gastric mucous membrane deep purple, mucosa of large intestine throughout intensely congested, of deep purple color, but without ulceration; one lung is congested. All other organs show no marked changes. Smears from spleen show anthrax like bacilli, probably anaerobic post-mortem growth. Culture tests from spleen negative. Guinea pig 188 inoculated from lung, died in two days of swine plague bacteria

infection. Guinea pig 187 inoculated from spleen, died in twelve days but yielded no cultures nor showed any lesions referable to swine plague infection.

Cultures from pig 188 when tested on other laboratory animals showed a high degree of virulence, 0.1cc. of a broth culture being fatal to 400 gram guinea pig in thirty-six hours.

Hog 12.23.04, which had been dead over 12 hours, when opened was found too much decomposed for profitable dissection. All the abdominal viscera were infiltrated with gas. Lungs were deeply congested; fat and subcuta punctuated by blood extravasates. Bowels seemed to be free from all ante-mortem change. Culture or inoculation tests were not attempted.

Hog 12.30.04 showed a purple hemorrhagic condition of large gut except rectum which appeared normal. No thickening or sign of ulceration, other abdominal viscera not obviously altered. Anterior three-fourths of both lungs solid and adherent by fibrinous exudate to thoracic walls.

Cultures showing morphology of swine plague bacteria were obtained directly from spleen, but when inoculated to a guinea pig produced no obvious effect.

At this visit, besides the above animal, four others were lying around in various stages of decomposition. One of these, a small sow which had not been on exhibition, when opened showed pneumonia as above, and no marked intestinal changes.

Hog 1.18.05 which had been sick for two or three weeks died during night; opened at 11 a. m., while still warm, by assistant, and spleen brought to laboratory at 2 p. m. Lungs are both consolidated, pneumonia typical of swine plague, no pleurisy; spleen somewhat enlarged, discolored and softened; liver light colored and flaccid, degenerated; kidneys not on hand. Cecum shows three ulcers, two about 1 c. m. diameter with yellow coating and no thickening, and one large ulcer close to ileo-cecal valve. None of these are typical of hog cholera. Mucous membrane generally not otherwise changed, except for one hyperemic patch; rest of gut not brought to laboratory was reported normal.

Bacteriology.—Culture test from spleen and liver yielded no growth. Covers from spleen and liver showed large round-ended rods (anaerobic); from lung vacuole staining ovals like swine plague bacteria.

From this hog the following inoculation tests were made:

Guinea pig 192 from spleen, without effect; guinea pig 194 from lung, without effect; guinea pig 193 from scraping from cecal ulcer, died on second day, yielding cultures of swine plague bacteria.

Pig 1.18.05a, received in subcuta of thigh 2 c. c. of mixed blood from spleen, liver and lung. It died in two weeks with small local abscess and lobular patches of pneumonia (red hepatization) scattered through right lung, the left being normal. Both ventricles contain yellow clots. Other organs unchanged. Culture test from viscera negative.

The effect of this inoculation was slight, localized in the lung and probably would not have proved fatal but for the contributory effect of extreme cold weather to which the animal was subjected.

Considering this outbreak as a whole, its most prominent features are: (1) absence or nearly so of ulcerative intestinal lesions; absence of hog cholera bacilli; pneumonic lesions in greater or less degree in all; acute hemorrhagic intestinal lesions in some; the uniform presence of swine plague germs either generalized or localized in the pneumonic areas. The diagnosis, from the pathologic changes and bacteriological finding, would be that of Smith's swine plague, or according to later views, perhaps, hog cholera (by infection with filterable virus) complicated with infection with swine plague bacteria. It differs from outbreak 2 in the absence of intestinal ulcers of the typical hog cholera form, but resembles it in the absence of *Bacillus cholerae* and presence of swine plague bacteria.

The significance of the presence or absence of each of these pathogenic species in our prevalent infectious swine disease may be better considered after the description of another outbreak which closes our record of investigation of naturally acquired infection.

Outbreak 6.—Patho-anatomical changes of hog cholera and swine plague, conjoined or separate; bacterial infection with swine plague bacteria, or *Bacillus cholerae suis*, or negative.

As in many other outbreaks the original source of infection in this case could not be accurately traced. The stock consisted mainly of brood sows and pigs with a few shoats and one large male, all of mixed breeds, grades or crosses of Poland China and Tamworth. No disease on the farm till the middle of March. About the first of March a sow which had been brought to the place by a farmer, for mating, was observed to be weak and stiff in gait when removed, but

recovered later. This farmer had no disease on his place then or later. A few days later the boar became sick and by the first of April most of the hogs on the farm were lying around more or less sick, and deaths began to occur in rapid succession.

Another theory was that infection occurred from the slop from the college dormitory which was regularly fed to these hogs. In this slop pieces of uncooked pork which had been rejected and thrown into the waste barrel were supposed to come from infected hogs. A fact in support of this theory is that at least two other nearby farmers who had at different times hauled and fed this dormitory refuse, also obtained disease in their hogs which in one case we found to be hog cholera.

Most of the dissections in this outbreak were made by assistant veterinarian J. F. Stanford, and material brought to the laboratory for bacteriological study. The symptoms given were: Eye in some mattering; yellowish thin feces passed by some; in others dissected there was constipation with hard balls in large intestines. Coughing was not much observed; some, including all of the young pigs, showed "*thumping*;" weakness in hind quarters marked in all. Temperature in two sick hogs was found to be 105° F. The following extracts from the records show the principle data obtained from the investigation of this disease.

Hog 3.27.05, weighing about 100 pounds, had been sick about a week; died in morning; autopsy at 3 p. m. Peritonitis with much membranous adhesions; surface of gut a dirty grey and much fluid of similar color in peritoneal cavity. Spleen not enlarged. Liver and kidney not noticeably changed. Lungs contain a small area of pneumonia (red hepatized). An ulcer, size of a quarter, with thick base, in stomach; mucous membrane of intestine apparently normal.

Culture tests from liver and spleen yielded growths of *Bacillus coli* only.

Guinea pig inoculated from scraping from liver and spleen died in two days with putrid exudate, dissecting the skin of side from body. No marked peritonitis. Cultures gave mixed growth from which nothing was learned.

Pig 3.29.05, six weeks old, the dam of which is also sick. Small patch of red hepatization in each lung; liver large, congested; spleen dark, but little enlarged; kidneys show no obvious change. In large

gut close to ileum a number of small ulcers close together cover a space the size of a quarter, each covered by a dark adherent slough.

Culture tests from liver and spleen—negative.

Hog 3.29.05a. Engorged lymph nodes, hemorrhagic spots on serosa of small and large intestines; bilateral pneumonia; several large ulcers in the large intestines.

This hog had been sick about two weeks.

Guinea pig inoculated with scraping from cecal ulcer without effect.

Pig 3.29.05b. Hemorrhagic spots on serosa of intestines; numerous ulcers in large intestine and some in small; pneumonic area in one lung. This sow had been sick about ten days and had been observed to pass blood in feces. The ulcers in large intestine, when examined in laboratory, were such as are found in cholera.

Culture test from liver and spleen—negative.

Hog 3.31.05, a small Tanworth pig. Engorged spleen and lymph nodes, small pin ulcers in cecum, general enteritis, limited pneumonic patch.

Hog 3.31.05a, an eighty-pound red shoat, in fair condition. It was unable to stand on hind legs and apparently near to death. Killed for examination.

Autopsy.—Purple color along ventral surface of abdomen; considerable yellow peritoneal fluid; one large, dark ulcer with thick base in cecum; spleen dark, but little enlarged. Kidneys show on surface several deeply reddened spots 1 c. m. or less in diameter; about half of one lung hepatized and presenting the usual swine plague purulent pneumonia. Colic and other lymph nodes swollen and blood engorged.

Bacteriology.—Culture test from spleen, liver, lymph nodes and lung, all positive. None of these cultures showed *Bacillus cholerae* but seemed to be swine plague bacteria. That from the liver subjected to further biological test confirmed this diagnosis.

Inoculations.—Guinea pig 190, from colic lymph node died in ten days, yielding cultures of swine plague bacteria.

Guinea pig 190a, previously immunized against swine plague bacteria; inoculated from spleen, remained unaffected.

Guinea pig 174a, previously immunized against hog cholera bacilli, inoculated from lung, died in two weeks, with pleuritic and pericardial adhesions, and yielding cultures of swine plague bacteria.

Pig 3.31.05b, small ulcers in cecum, hemorrhagic infarcts in lung.

Pig 4.1.05. Inflammatory changes in intestines and stomach, with commencing ulcers in cecum; hemorrhagic infarcts in lung.

Hog 4.9.05. Purple color along ventral surface of abdomen; glands of groin greatly engorged, congestion of spleen, liver and kidney. Cecum and adjacent parts of colon show many ulcers from the size of a dime down, black on surface and with thick base; further up smaller ulcers are forming. These ulcers might be considered typical of hog cholera.

Culture test from lymph node of groin gave a growth of streptococci; from liver a mixed growth of swine plague bacteria and *Bacterium coli.*

Guinea pig inoculated from cecal ulcer and from engorged lymph gland of mesentery, died in fifteen days without marked lesions, and a bacterial finding of bacilli (culture test from spleen and liver), which were found to give the reaction of the colon group in the case of the liver and *Bacillus cholerae* in those from spleen.

Hog 4.10.05, a large sow, opened soon after death, and specimens brought to laboratory. Said to be perforation of bowel, and offensive fluid in peritoneal cavity. Liver is large, congested, and shows many light colored small necrotic-looking areas on surface. Spleen little if any enlarged, but very dark and solid to knife; lung congested; some ulcers, mostly of small size, in large intestine; small intestine very dark red in color, almost phagedenic in appearance; lymph nodes everywhere congested; many of those in the mesentery seem to be necrotic in center.

Cultures from liver and spleen both give growth of *Bacillus cholerae suis.*

Hog 4.11.05, pneumonia and cecal ulcers.

Pig 4.12.05, purple color of belly; engorged lymph nodes; congestion of mucosa of stomach and large gut; red hepatization in anterior lobes of lungs.

Culture tests negative.

Hog 4.12.05a, weight about sixty pounds. Typical swine plague pneumonia, fibrinous pleuritis, numerous ulcers, large and small, throughout the large intestine.

Cultures of swine plague bacteria obtained from spleen.

Hog 4.12.05b. Lungs congested; numerous ulcers in cecum, engorged spleen.

Guinea pig 190a, previously immunized against swine plague, inoculated from spleen. It died in eleven days and yielded cultures of bacillus cholerae from liver and spleen.

Hog 4.15.05. One deep pit-like ulcer, and many large, rough, inflamed areas in cecum; consolidation of large part of one lung, spleen enlarged and dark; large hemorrhagic area in leaf lard.

Cultures show a mixed growth giving a gas reaction of *Bacillus coli.*

Hog 4.15.05a. The record of autopsy on this hog was lost. Culture tests from the spleen of this animal gave a mixed growth including *Bacillus pyocyaneus.*

In order to test the contagiousness of this disease two shoats previously unexposed were put into the field where these hogs were dying. In two weeks both were obviously sick. A third introduced later also became infected and died one month later.

Dissections were also made of these hogs.

Hog 5.17.05, which had been visibly sick for a month died on this date. It showed a large sore exposing bones of joint on carpus of one leg; lymph nodes and lung appeared normal; liver shows pale areas of fatty change; spleen dark, soft and slightly enlarged. Kidneys normal. Cecum has mucous membrane mostly covered by a yellow scurf partly attached and partly loose; also ulcers with thick base especially near ileum opening. Other parts of large intestine show same false membrane in patches and also round or oval slightly elevated areas about 1 c. m. in diameter, some of which have the same yellow surface coating. These seem to be commencing ulcers.

Culture tests from liver and spleen yield growths giving the fermentation tube reactions of the colon group of bacteria.

From this hog inoculations were made of 2 c. c. of heart blood subcutaneously, to another hog not previously exposed. This hog designated 5.14.05a, was obviously sick by June first, that is, in two weeks after inoculation. It died on June 14th, and was dissected on same day. The pathologic changes were confined to the thoracic organs, the greater part of the left lung and part of the right were consolidated and studded on surface and on section with yellow spots, recalling the yellow nodules of porcine tuberculosis. This is typical of advanced stages of pneumonia of swine plague origin.

Culture tests from abdominal viscera were negative.

Besides the three pigs above referred to, twelve others were introduced into this infected field while the disease was still prevailing.

These were introduced for the purpose of testing a method of vaccination which will be later referred to. They were left in this field for ten weeks by which time eight had died, the other four being apparently well. In those that died the conditions found on post-mortem examination were similar to those already given and all of them need not be further described. From one of these which on section showed a yellow necrotic exudate on the mucosa of the large intestine subcutaneous inoculation of 1 c. c. of blood from heart was made to another pig weighing about sixty pounds, which was put in a clean and isolated pen. It showed sickness on the eleventh day following inoculation, and died on the twenty-ninth day. There was extensive pneumonia involving the posterior half of both lungs, and also fibrinous pleuritis. The cecum was covered by elevated, button-like ulcers typical of hog cholera.

Culture tests were made from the spleen yielding growths of swine plague bacteria only.

At the same time this pig was inoculated a guinea pig weighing 371 grams, previously immunized against swine plague, received subcutaneously 0.3 c. c. of heart blood as used on the pig. The result was negative, the animal remaining unaffected.

In this last lot of pigs there was the same variability in the prominence of pulmonic or intestinal lesions as in those already described and also the same variability in the bacteriology.

Bacillus cholerae was not isolated from any in this lot, swine plague bacteria from some, and negative results in others.

In reviewing the records of this outbreak the most striking features may be summarized thus: Although all were alike exposed, some showed most prominently the intestinal changes which are generally considered to be associated with cholera, others pulmonic lesions typical of swine plague; in some both of these were present in about equal prominence. The intestinal changes in different animals varied from typical button ulcers with thickening and rigidity of the wall of the gut to superficial necrosis or acute hemorrhagic enteritis. Swine plague bacteria were apparently present in all the pneumonic lesions, and generalized in a certain number independent of the character of the intestinal lesions. It will be noticed that in some in which the cecal ulcers were most typical of cholera, swine plague bacteria were alone obtained. In some animals showing pronounced anatomical changes, bacterial tests from the abdominal viscera or

inoculations of small animals proved entirely negative. In three cases in which *Bacillus cholerae* were found one was obtained by large seeding from the spleen, and another in a mixed growth from the liver. This bacillus was obtained by animal inoculation once only, and then by the use of a guinea pig immune to swine plague germs. It was apparently possessed of little virulence. However, it gave a positive reaction with hog cholera agglutinating blood and gave the fermentation reaction proper to *Bacillus cholerae*. It was considered as a variety of the hog cholera bacillus, but probably a factor of little importance in the etiology of this outbreak.

It will be noticed also that the inoculation tests from hog to hog gave in one case swine plague pneumonia as the prominent feature, and in another pneumonia conjoined with characteristic intestinal ulcers.

THE SPECIFIC ACTION OF BACTERIA IN HOG CHOLERA.

From the foregoing records it would be impossible logically to refer the intestinal lesions of hog cholera to the action of the *Bacillus cholerae suis* only. They might with equal or more reason be attributed to the action of swine plague bacteria, since these were present with much greater uniformity. Of the pathologic changes referred especially to swine plague as an independent disease the thoracic lesions, pneumonia and pleurisy, would seem to be almost certainly due to the action of the bacteria which go by that name, acting either independently, as in cases described in outbreak 3, or, in the more contagious forms of the disease, possibly as a sequel to a primary infection with the filterable virus of Dorset and Niles.

Further evidence of the specific action of these bacterial species may be obtained by direct infection experiments with pure cultures. Inoculation and feeding experiments with cultures of cholera bacilli were made by Smith with results which were at least not contradictory to his view that this organism was specific in hog cholera.

This part of the bacteriology of hog cholera, however, has not received as much attention as it requires, and we present here the records of our own tests in feeding and inoculation of hogs with cultures as a contribution to the subject only, the results as will be seen being hardly satisfactory in either proving or disproving the specific nature of the hog cholera bacillus in the etiology of the natural disease.

INFECTION BY INGESTION OF BACILLUS CHOLERAE CULTURES.

Our earliest experiments in this line were carried out in 1899, and the present description is largely an abbreviated reproduction of the notes of the experiments made at that time, hitherto unpublished.

The culture used in this test was obtained from a pig dead from natural infection in the outbreak referred to in the preceding pages as No. 1. It was derived from a single colony on an agar plate seeded from the spleen, then transferred to tubes of nutrient broth, and fed after three days growth at 36° C.

Two pigs, numbered 5 and 6, about three months old, were used in the first test. They were placed in a new pen and two others of the same lot placed in an adjacent pen to serve as checks against error arising from a possible prior or extraneous infection.

January 13th, 20 c. c. of broth culture fed in milk.

January 17th, 15 c. c. of similar culture fed. It was taken reluctantly, the pigs being evidently sick from the first dose.

January 18th. Pig 5 died, five days after first infection.

Autopsy.—A black pig; skin between fore and hind legs where color is lighter is of a deep red. Lymph nodes of groins, arm and maxillary region congested. Liver shows no marked change. Spleen moderately enlarged and darkened. Kidneys show hemorrhagic points on capsule; on section, congestion with dark red discoloration of mucous membrane of pelvis. All the abdominal lymph glands are large and blood engorged, the gastric being almost tar-like on section. Heart surface blood streaked; lungs mottled with deep red in large areas, some lobules being almost black and solid to the knife. Stomach and intestines show nothing externally. When opened the gastric mucosa is deep red on pyloric half; small intestine, in first and last parts especially, shows blood extravasation. Cecum and colon throughout are deeply congested with, in places, beginning formation of false membrane; last part of colon appears almost gangrenous, friable and readily tears on manipulation.

Pure cultures of hog cholera bacilli were obtained in tubes from liver, spleen and kidney, impure from the lymph nodes of the lumbar region. Hog cholera bacilli colonies were also obtained on agar plates from the lung.

Rabbit inoculated from this culture dies in four days, yielding, in turn, cultures of bacillus cholerae only.

Pig 6 died on the eighth day after the first dose. The post-mortem findings were similar to those of pig 5 in every respect, and pure cultures of hog cholera bacilli recovered from the abdominal viscera.

The lung lesions or infarcts, when examined in section, showed intense blood extravasation into the air vesicles and minute bronchioles.

The check pigs numbered 7 and 8, which had remained in health, were, on March 3, put into the same pen where Nos. 5 and 6 had been confined, and on same date two other pigs were added to this pen.

These were fed 25 c. c. of broth culture of cholera bacilli of same origin as before, being the fifth transfer from the original. The tube *cultures used were of five days' incubation.*

On March 8th these four pigs received another dose of 20 c. c. broth culture.

Pig No. 7 was apparently sick at this time and died three days later. Section revealed discoloration (without consolidation) of one lung, the other being normal. Cecum and greater part of colon on mucous surface covered by a rough, adherent, necrotic coating. Other conditions as in pig No. 6, except that the lymph nodes generally were less markedly engorged.

March 13. The three remaining pigs were fed 20 c. c. of fluid culture as before, and on March 24th this dose was repeated.

Pig No. 8 died March 29th. Besides the lesions already described in the other the cecum and first part of colon contained isolated ulcers as well as patches of adherent exudate.

The two remaining pigs, Nos. 9 and 10, which were of larger size, remained apparently unaffected.

April 13th two more were added to pen and the four fed 80 c. c. broth of the same origin as before, but now ten weeks old and several transfers from original animal. No effect was observed following this dose, but all of these pigs when dissected four months' later showed small isolated superficial ulcers in the cecum in greater or less number.

Second Test.—This was carried out in 1902, as a part of some experimental tests of attenuated cultures in protecting from infection with virulent cultures.

Six pigs about four months old, three of which had been previously fed cultures of naturally feeble virulence were placed in one pen and fed broth cultures of *Bacillus cholerae* from two independent sources. These cultures were originally of the usual degree of virulence and exhibited no attenuation when tested on laboratory animals. They had been in artificial culture for a year or more, and apparently had lost virulence for hogs. To these six pigs fluid cultures of four days' incubation were fed on two occasions at intervals of one week, the dose in each case being 80 c. c. A temporary loss of appetite followed the first dose, but this was the only effect produced. As all remained in apparent health no post-mortem examinations were made.

During the course of our experiments in preventive vaccination by attenuated cultures, tests of the protection afforded were frequently made by feeding virulent cultures. These tests were, however, carried out in infected pens so that the results obtained could not, with certainty, be ascribed to the action of the ingested cultures. Where, in some cases, pen infection could be excluded by reason of early death and dissection the lesions found were essentially as described above. Isolated ulcers when present were more superficial than in the natural disease. More frequently the intestinal lesions were in the form of continuous or extensive superficial bran-like exudates sometimes involving parts of the small intestine as well as the large, and more rarely the gastric wall and pharyngeal region also.

More recently we have had occasion to repeat these experiments of infection by ingestion in order to test the contagiousness of this disease occasioned by the hog cholera bacillus, and compare the pathologic condition with that induced by blood inoculations.

The method as outlined was to feed fresh virulent cultures of *Bacillus cholerae suis* as obtained from blood-inoculated hogs (during the process of preparation of hyper-immune serum) and place with the pigs thus treated others untreated, in order to observe if fatal disease was thus transferred. The natural disease being highly contagious by cohabitation in pens should be imitated in this respect by the bacillar disease induced by artificial infection if the two conditions were identical.

1907. Dec. 20th. A thirty-pound pig, which, for convenience, is designated No. 1, was put in a new pen about six by eight feet large, covered and floored. It was fed, after fasting the previous

evening, 20 c. c. of culture of the following record: It was got from the spleen of hog 12.8.07, which had died from inoculation of blood of hog cholera received from the government hog serum station in Iowa, by growth on slanting agar tube, transferred from one colony to a second agar tube, and from this to nutrient bouillon, all at three days interval. The purity and identity of culture was assured by all necessary biologic tests. The culture was fed in a quart of milk and all taken.

Dec. 21, 20 c. c. more of similar culture was given.

Dec. 22. Pig No. 1 has an offensive thin whitish fecal discharge, but does not appear sick. Into this pen was introduced pig No. 2, weighing about sixty pounds, the trough in which the feeding had been done being previously removed.

Dec. 24. Pig No. 1 died and was removed for dissection.

Dec. 29. Another check pig (No. 3) was put in pen and on the 30th pig No. 4, which, the previous day, had been fed 60 c. c. of culture in a separate box, was also added to this pen.

Jan. 3d. Pig No. 3, untreated, died and was removed for dissection. The other two seemed little inclined to eat.

Jan. 6. Pig No. 4, which had been fed the culture 8 days before, was found dead. The remaining animal No. 2 (untreated) remained in health and was removed from pen Jan. 20th.

Post-mortem Examination.—Pig No. 1 (12.24.07). There is marked reddening along venter surface, and on inside of hind legs. Lymph nodes of neck are darkened. Spleen is slightly enlarged and darkened; lungs discolored in places, but not inflamed. Stomach shows some yellowish exudate on mucous membrane easily detached, showing an apparently eroded surface beneath. Cecum is coated by an abundant yellow exudate also easily detached. Same condition exists in patches in the colon and last part of small gut.

Cultures of *Bacillus cholerae* were obtained in purity from spleen and liver.

Pig No. 4 (1.6.08a). This was the second pig which had been fed the bacterial culture.

Small white runt. No discoloration of skin, no appreciable engorgement of superficial lymph nodes. Spleen slightly discolored and softened. Small patch of congestion at apex of one lung. Cecum and part of colon show a yellow, slightly adherent exudate which

leaves an eroded surface when detached. At one part, in cecum, this exudate is in islets and suggests the beginning of the usual hog cholera ulcers. Other organs not mentioned show no appreciable change.

Culture of bacillus cholerae obtained from spleen.

Pig No. 3 (1.3.08) untreated. A white pig in fair condition. There is reddening of skin over belly and sides, and to less extent of the whole body. Skin everywhere, but thicker in some places than in others, studded with black spots which at first sight look like blisters, but when cut appear to be circumscribed blood extravasates. They are generally circular and from a half to one centimeter in diameter. Lymph nodes of body slightly darkened. Spleen not discolored, and but slightly enlarged. Kidneys show punctiform discolorations on surface. When cut and examined by lens the glomeruli stand out as red points. Stomach shows large, irregular, dark red extravasates of blood into the mucous membrane on greater curvature near the pylorus. Some of these dark patches show loss of substance like inflamed ulcers. Intestines and other organs not mentioned are not appreciably changed.*

Culture test from spleen yields growth of bacillus cholerae.

So far as the test of contagiousness is concerned, this experiment proved inconclusive in its results. One pig was apparently unaffected by the exposure, and the other, although it seemed to die from a generalized bacillar infection, showed lesions entirely dissimular to those usually met with in hog cholera. Another attempt was, therefore, made to solve this apparently simple problem of the contagiousness of the bacillar disease.

1908. Jan. 20. Into a previously unoccupied pen were placed four pigs of ages from two to four months, and in weight from thirty to seventy pounds. These were culls from the college breeding stock and not a very thrifty-looking lot of pigs, but the best available for this work.

In a small cage a three-months-old pig in good condition was fed 6 c. c. of a three-days' broth culture of hog cholerae bacillus, of same stock as previously used. This was the sixth transfer from hog 12.8.07. Next morning this pig (called No. 5) was put in the pen

*Sections through these skin lesions show the epidermis intact and masses of extravasated blood cells with scattered particles of blood pigment and engorgement of the capillaries and minute vessels in the corium. In these sections bacillus cholerae could not be demonstrated.

with the four untreated pigs. The same evening pig No. 5 seemed little inclined to eat and by the third day was obviously sick and refused all food.

January 24 another small pig (No. 6), which on the two preceding days had received 6 c. c. of culture (12 c. c. in all), was placed in pen which now contained six pigs, two treated and four for exposure test. This last pig (treated) also lost appetite on the first and following days after treatment.

January 31 a third treated pig (No. 7) was introduced. It had received 10 c. c. of culture on preceding day. Also, there was added another check or exposure pig.

The object in introducing infected pigs on different dates was to prolong the exposure period as well as to make sure of obtaining a fatal disease in the treated pigs.

On this date, January 30th, pig No. 6 was found dead in morning, having died during the night. It had been fed cultures eight days before. (For autopsy see forward.) Blood from heart was injected subcutaneously, in doses of 2 c. c., into another pig (No. 8) weighing about forty pounds, and this also put in pen with the others.

February 2. Pig No. 5, the first treated pig in this experiment, died, that is, thirteen days after first infection.

February 10. One of exposure pigs sick and refused food and another, a small unthrifty runt, also off-feed. A heavy snowstorm, with cold weather, occurred on the 15th, which finished this last-named pig. Its dissection is given under the name 2.15.08. The others seemed to recover and by February 20th all appeared to be in good appetite.

March 2. Pig No. 8, which had been inoculated with blood from No. 6 on January 31st, and which always seemed unthrifty, was found dead.

Post-Mortem Examinations.—Pig No. 6 (1.30.08), which died eight days after ingestion of hog cholera culture. A small black pig. It showed no external or internal pathologic features except as here noted. There was a yellow patch on serosa of gut looking as if bile stained; part of colon slightly blood-shot; mesenteric glands greatly enlarged (hypertrophied) and yellow in color. (This is a condition we have often found in unthrifty pigs, or "runts" as they are called, and not referable to hog cholera infection.) Cecum and part of

colon shows yellow, adherent exudate in patches, and some superficial ulcers. Lungs show areas of discoloration, but no pneumonia. Spleen hardly perceptibly enlarged.

Liver and spleen yield cultures of *Bacillus cholerae*.

(Blood from this animal was used to inoculate No. 8.)

Pig No. 5 (2.2.08). Died thirteen days after ingestion of culture. Marked red discoloration of skin of belly. Cecum shows thickening of wall and on mucous surface a thick yellow exudate continuous or in patches. Where isolated and washed off there is shown superficial ulceration or erosion of mucous membrane. In other places the exudate is ridged or uneven and firmly attached. In one patch the surface is dark in color. Mesenteric glands as in last animal. Liver shows light-colored areas and numerous purulent points, which when incised are seen to be definitely walled cysts with fluid or semi-solid contents. This is regarded as a parasitic lesion and not referable to the inoculation.

Culture tests from liver give a mixed growth which includes a green chromogenic species. From spleen a growth containing a motile bacillus which does not give the fermentation test of *Bacillus cholerae*. Hog cholera bacillus was not isolated from this animal.

Pig 2.15.08. This was a small runty pig used as a check or exposure animal. It was never thrifty and died during a spell of severe winter weather which was thought to be the main cause of death. However, the dissection showed that this animal was also infected. The kidney which appeared normal on surface showed a marked abnormality on section, the cortex being pale and homogeneous and pyramids a deep red projecting above the cut surface. On microscopical examination of sections this condition was found to be due to blood extravasation into and around the tubules. Spleen slightly engorged. Cecum shows a few yellow prominent exudates on mucous surface which leave depressions when washed off. Much of colon is in same condition, but in part of it they are much more numerous, generally about the size of a pin-head and rather firmly attached.

Cultures from spleen and liver consist of motile bacilli which correspond in all biologic features to hog cholera bacilli.

Pig No. 8 (3.2.08) inoculated with post-mortem blood of No. 6 on January 31st, died March 2d, after a lengthy period of unthrift. Body thin, skin not discolored and no local lesions at place of inocu-

lation. Arborescence of omental connections of large gut. Cecum shows no abnormality. The colon contains on mucous surface a number of flat, projecting white patches which look like healed ulcers, but cannot with certainty be diagnosed as such.

Culture test from spleen yields a growth of bacillus cholerae.

Agglutination test of blood with culture of *Bacillus cholerae* is positive although the reaction is slight.

Summarizing the records of this experiment we find that of the three animals fed with cultures of *Bacillus cholerae* in doses from 6 c. c. to 12 c. c., two died with the usual lesions referable to this infection; the third never showed sickness.

Of the five animals exposed but untreated, one died and one became sick and recovered. The pig that died presented post-mortem conditions similar to those infected by feeding.

One pig inoculated with blood from an animal dead from ingestion of cultures died with dissemination of bacilli in the abdominal viscera, and with intestinal lesions presumably referable to the inoculation. The bacillar-induced disease, therefore, is contagious or transferable by cohabitation, and is also transferred by blood inoculation, but, in both respects, much inferior to the natural disease called hog cholera. This, at least, is the deduction afforded by these two tests.

Feeding of hogs with the carcasses of animals dead from artificial infection with *Bacillus cholerae* does not produce any more virulent diseases than cultures in vitro. Laboratory animals dead from inoculation with this bacillus have frequently been fed in the effort to maintain or enhance the infection in our exposure pens in connection with vaccination experiments, the results being about the same as by the use of cultures on artificial media.

Inoculation of Cultures.—This mode of infection, when the inoculation is subcutaneous, has been regarded by other investigators as uncertain in its results. In numerous tests which have been made here in connection with vaccination experiments we have had this experience. Large hogs generally have shown no effect from inoculation of the same cultures which proved fatal when ingested. In a few cases, however, we have obtained fatal infection in this way by the use of virulent cultures on young pigs, and even with attenuated cultures have obtained intestinal ulceration of a mild type. After inoculation of cultures the blood of the animal in the majority of

cases acquires agglutinating properties for cultures of *Bacillus cholerae*. The intestinal lesions observed after inoculation of cultures of naturally feeble virulence are in the form of very small and scattered elevated nodules on the mucosa of the large gut. On surface these show erosions and may be covered by a slight exudation similar in appearance to that produced by ingestion infection. We have never observed that such inoculated animals are capable of disseminating disease to others with which they cohabit. In the few cases of fatal infection by the use of virulent cultures there was found a condition resembling that produced by feeding cultures, but the superficial necrotic areas in the intestines were smaller in area and in isolated patches. *Bacillus cholerae* can be obtained in cultures from the abdominal viscera.

Intra-peritoneal inoculation of cultures begets a peritonitis and muco-enteritis of the usual type as may be seen from the following case taken from the records:

Pig 1.22.01, weighing about seventy pounds, received in abdominal cavity 6 c. c. of a bouillon culture, twenty-four hours old of hog cholera bacilli. It died nineteen days later and showed the following condition post-mortem:

Severe peritonitis with extensive adhesions, thickening of mesentery and considerable turbid peritoneal fluid. Spleen little enlarged, but buried in adhesions; liver congested and bile thick yellow and granular. Large intestine contains several ulcers as well as much adherent necrotic exudate in small and large patches which show erosions of mucosa when detached; last foot or so of small gut in same condition. Lungs are little changed, but there is membranous adhesive pleuritis of large extent.

Cultures of *Bacillus cholerae* were obtained from liver. Guinea pigs inoculated from cecal ulcers and pleural exudate died in eight days and two days, respectively. From the former hog cholera bacilli were obtained, and from the latter a mixed growth of hog cholera and swine-plague germs.

From the foregoing experiments it is hardly disputable that all the anatomical lesions found in natural outbreaks of hog cholera may be reproduced by artificial infection with pure cultures of *Bacillus cholerae suis*—erythema of ventral surface of body, punctiform hemorrhages on serosa of abdominal organs, engorgement of lymph nodes and spleen, congestion or limited areas of consolidation

in lung, and inflammation with superficial necrosis or ulceration on intestinal and gastric mucosa. On the other hand, the artificially induced disease has not been shown to have the same degree of contagiousness as the natural disease.

The pathogenic action of *swine plague bacteria* on hogs when infected by artificial cultures is a subject which requires further study. We have made few infection tests with this species except on laboratory animals. In rabbits and guinea pigs the most characteristic lesion resulting from inoculation is a general peritonitis with enormous masses of bacteria demonstrable on the inflamed serosa. The bacteria are also generalized and recoverable in cultures from all the internal organs. Pigs inoculated in the lungs or peritoneum die from pleuro-pneumonia or peritonitis, but subcutaneous inoculations seem to be generally ineffective. So far we have made no experiments of infection by ingestion with swine plague bacteria. Our bacteriological studies of outbreaks of hog cholera, as the above records show, seem to indicate that the intestinal lesions produced by this species are entirely similar to those produced by the *Bacillus cholerae suis*. That they are responsible for the extensive pneumonia often seen in natural cholera outbreaks can hardly be doubted. The presence of these germs generalized throughout the body in outbreaks of hog cholera (as commonly called) with typical intestinal ulcers and at the same time the absence, so far as can be shown, of *Bacillus cholerae* naturally suggest the thought that they are the cause of these lesions in such cases, but such an assertion would not be warranted without the evidence to be derived from infection experiments with cultures.*

The Filterable Virus of Hog Cholera.—It has been shown that the pathologic features of hog cholera can be produced by infection with

*The hog cholera ulcer in its earliest stage we believe to be represented by the minute elevations with eroded surface found after subcutaneous inoculation with attenuated hog cholera bacilli. These in vertical sections show a loss of substance in the superficial layer of the mucous membrane. Although such ulcers have been regarded by some as embolic in origin these slight lesions do not convey this impression. The submucous tissue at this stage is but slightly infiltrated with round cells. There is exfoliation of the epithelia between and surrounding the mouths of the gland tubules, engorgement of the capillaries immediately subjacent and exudate of fibrin on the surface. In this is contained the shed epithelia. Those most deeply seated still take the nuclear stain, but those nearest the surface of the exudate have undergone complete necrosis and do not stain.

This also is the histologic structure of the exudation found on the mucosa after ingestion of cultures.

In older ulcers the mucous membrane is completely lost and the walls enormously thickened by the usual products of productive inflammatory action.

The yellow consistent exudate frequently seen in the natural disease and constantly present after artificial infection by ingestion of cultures is not due to any specific action of either *Bacillus cholerae* or swine plague bacteria, since we have at times seen the same condition produced by the biting worms of the small intestine when these were present in large numbers.

the *Bacillus cholerae* and that these are also frequently associated with, if not caused by, the swine plague bacteria of Smith. But Dorset's investigations, which have not been successfully disputed, show that hog cholera can be produced by inoculation of material which contains neither of these germs. The full explanation of this fact must await further research along the lines which we have attempted to follow in the investigations here reported. The simplest theory readily suggests itself: That the invasion of one or both of these bacterial species is dependent upon an abnormal condition of the system of the animal induced by the action of the filterable "virus" which is, therefore, the actual cause or initiator of the disease. On this theory the *Bacillus cholerae* like the *Bacterium coli* should be present in the animal under normal conditions, and act as a pathogenic agent only when the conditions are prepared for it. The same reasoning should apply to the swine plague bacterium which Moore has shown to be actually present on the mucous surfaces of many animals under normal conditions. In this respect there is a close analogy with the *Diplococcus pneumoniae* of man which, though bacterial in nature, is frequently sporadic and dependent for its initiation on external causes, but at times highly contagious by close cohabitation. Virulent forms of *Bacillus cholerae*, however, have not been found except in association with hog cholera.

According to the records of the experiments of Dorset and Niles, animals which die from inoculation of filtered blood may or may not yield cultures of *Bacillus cholerae suis* post-mortem. In our inoculations with blood, which was not filtered, during the preparation of serum, *Bacillus cholerae* was nearly always obtained in cultures from spleen or liver in acute cases. It is probable that in most instances the bacillus was introduced with the inoculated blood although this would be unnecessary if it was a constant inhabitant of the hog according to the theory just referred to. The failure to find *Bacillus cholerae* in any case is not an absolute proof of its absence during the earlier stages of the disease. In our inoculations above referred to (in the preparation of serum) the blood obtained from one hog dead from natural infection and also from other hogs killed with this blood, though possessed of the usual degree of virulence, rarely allowed of a demonstration of bacilli in animals to which it was inoculated. Out of five such animals in which culture tests were made *Bacillus cholerae* was obtained in one only. With blood from other sources it was the exception not to obtain cultures. We infer

from this that in blood-inoculated animals the bacillus recovered from the viscera may have origin either in the inoculated blood or, according to the theory, from the intestinal tract of the animal, where it may be normally present.

Preventive Vaccination.—On the theory that hog cholera (and swine plague) were of bacterial origin much effort has been expended by various investigators in the production of a protective vaccine by the use of bacterial cultures but no vaccine prepared in this way has proved sufficiently successful to come into general use in this country. It is easily possible to vaccinate against hog cholera bacilli, and also swine plague bacteria, and the artificial disease occasioned by these organisms in small laboratory animals, but hitherto such modes of vaccination have not been proved to be of much practical use in the prevention of the natural disease in hogs.

Our work in this line included tests of anti-toxic serum prepared by the injection of bacterial cultures into cattle, vaccination by the use of killed cultures, and vaccination by the use of artificially or naturally attenuated germs.

Tests of anti-serum proved so unpromising that they never went beyond laboratory animals.

Sterilized cultures of *Bacillus cholerae* have been quite largely tested in our laboratory on small animals, and found to afford no protection against later infection with the living germ. After such injections the blood acquires agglutinating properties towards this bacillus, but no immunity is conferred. With swine plague bacteria the case is different. Here cultures sterilized by heat act as most efficient vaccines against inoculation with the living germ.

A summary of the results of our work in this line will suffice for this report.

Rabbits and guinea pigs which have received subcutaneously as little as one-half cubic centimeter of a broth culture sterilized by a brief steaming in the Arnold, obtain regularly a complete protection against a later inoculation with living cultures invariably fatal to check animals.

The immunity is acquired in one or two days after vaccination, and lasts for some weeks at least.

The vaccinating substance is contained in the bodies of the bacteria, and is not present in the fluid filtered through porcelain.

Vaccination against swine plague infection affords no protection against hog cholera bacilli.

Laboratory animals thus vaccinated we have found useful in the bacteriological study of hog disease where the presence of swine plague bacteria, which are often fatal to inoculated animals in from one to two days, may mask or completely prevent the discovery of the *Bacillus cholerae*.

Vaccination against infection by hog cholera bacilli we have found to be quite easily and successfully applied by inoculation with attenuated cultures. Artificial attenuation of virulence in *Bacillus cholerae* does not seem possible by any means which we have tried.

This species is extremely tenacious of its virulence under artificial cultivation, although strains or varieties of varying degrees of virulence occur in nature. We have found it equally difficult to increase the virulence of a naturally attenuated variety. The culture used in our vaccination experiments* was fatal to the guinea pig by intravenous but not by subcutaneous inoculation. In a series of inoculations designed to test the possibility of increasing the virulence of this variety it was passed by intravenous inoculation through twelve successive guinea pigs without any appreciable increase.

Inoculation with this attenuated variety was found to confer a certain and lasting immunity against inoculation with a virulent variety.

Tests of the value of this double vaccination (against swine plague by heated cultures and hog cholera by attenuated cultures) were carried on for several years on the rare occasions on which suitable opportunities occurred. In our own pens some favorable results were obtained, but where applied by others on the farms it met with little or no success. In the last test made during an outbreak on the college farm, twelve hogs, of which six had been thus vaccinated and six untreated, were exposed to the sick. The final result was that three of the vaccinated animals and five of the checks succumbed to the disease.

SERUM-SIMULTANEOUS VACCINATION.

During the past two years vaccination experiments according to the method of Dorset and Niles have been carried on at this station

*Obtained from the laboratory of Comparative Pathology, of Harvard, and isolated by Theobald Smith.

for the purpose of testing its efficiency and safety under the conditions prevailing in this State. We have attempted to ascertain the conditions under which failure of satisfactory results may occur and undertaken some investigations suggested by these experiments.

The *safety* of this mode of vaccination is manifestly a condition of vital importance if it is to be used in herds not already infected. In the use of a living virus as a vaccine there is, necessarily, a suspicion that it may under certain conditions beget a plague instead of suppressing one. A vaccine which has proved safe in its application by its originators, may be very unsafe when applied indiscriminately by farmers or even by veterinarians. In our first experience with the serum-simultaneous or "Government" vaccine this question of the safety in its application was the chief object in view.

Along with seventeen shoats varying in weight from thirty pounds to seventy pounds, which were to be vaccinated, three others were used as checks. The vaccine—blood and serum—was received August 20th, 1907, and kept on ice for three days before use on account of unsuitable weather. The vaccination was made according to directions, 2 c. c. of blood and 20 c. c. of serum, without reference to size or weight of animals.

The first symptom of disease appeared simultaneously in the pen containing vaccinated pigs and in that containing inoculated animals and the first death occurred September 9, seventeen days after treatment. This was a vaccinated pig weighing when vaccinated, about fifty pounds.

The final result of this test was that of the twenty hogs in this pen nine died within seven weeks after the date of vaccination. This included all the check animals (3) and six vaccinated pigs. The survivors were immune. They were exposed seven months later in a field containing cholera-sick hogs without contracting the disease while three out of four check pigs died from the same exposure.

The failure of this test was thought to be due to the fact that some of these pigs had been castrated shortly before vaccination (ten days), or to an insufficient dose of serum for pigs of the size used. From later experience we are now convinced that the latter was the true cause. The reported experiments up to that time had been made with pigs of a uniform weight, generally around thirty-five pounds, in which a standard dose of 20 c. c. of serum had proved sufficient. Our experience since that time has shown that the matter of dosage, in relation to weight, is of first importance in order to

avoid untoward results. Regulation of dosage necessarily implies standardizing of strength of serum in order to place this vaccination on a safe footing. An economical or accurate method of accomplishing this has not yet been evolved.

Later experiments which we have made with varying doses have shown that 75 c. c. of serum is about the maximum dose for hogs of any size, that is, of such serum as we have used, where 20 c. c. has proved sufficient for pigs weighing thirty to forty pounds. It would seem, however, that the dosage of serum required must depend also on the virulence of the blood used simultaneously with it, and this has been found to vary greatly. One of our chief difficulties has, in fact, been in keeping on hand a virulent stock of blood. It is practically impossible to collect blood in quantity without bacterial contaminating organisms.

Dried Blood.—Two separate experiments were made to test the virulence of blood which had been dried in thin layer at 36° C., immediately after collection. Inoculation with such dried blood, however, proved without effect, the virus or active agent in the blood having apparently been destroyed in the brief period of two days.

Our experience in the use of serum-simultaneous vaccination has been less favorable than that reported from other experiment stations, but we have no desire to condemn it. It is obvious, however, that in its present stage the preparation of the vaccine and its distribution should not become a commercial enterprise but remain under Government or State control.

Serum alone vaccination is on a different footing. It is safe, and its use need be restricted only by its cost and degree of efficiency. In our experiments in the treatment of cholera-sick hogs, it has proved quite useless, but has been used with some apparent success in preventing extension of disease in herds already infected. The problem here is to increase the potency of the serum and diminish its cost. We have attempted to accomplish this latter object by the use of cattle in the place of hogs as a medium in which to develop the anti-serum. For this purpose several young cattle were subjected to periodical subcutaneous injections of hog cholera blood after the manner employed in producing hyperimmune blood in hogs. It was found that these injections are not well borne. Enormous abscesses develop after each injection, accompanied by fever and rapid loss of

flesh. After a prolonged treatment the blood was not found to have acquired any protective properties when tested on hogs.

Mode of Infection in Hog Cholera.—The chief carrier of infection in cholera has generally been thought to be the fecal discharges. This was a natural assumption on the theory that the disease was essentially bacterial but unwarranted on the more recent theories of its etiology, where the actual cause is unknown. Two experiments in this direction were carried by Dr. J. F. Stanford, assistant in this work.

Transference by Lice.—A large number of these vermin which are sc common on hogs, and especially sick hogs, were carefully picked off from the body of a cholera-sick pig and transferred to that of a healthy pig which was kept under observation for a month. The result was negative.

Transference by Feces.—Fecal matter from the rectum of a pig dead of cholera, was mixed with slop and fed to a healthy pig in an isolated pen. The first test failed of result, but a second produced a fatal infection. A pen or field, therefore, becomes infected by the fecal discharges of sick hogs, and retains this infection for an unknown period. In a solitary test of the duration of infection positive results were obtained after one day and five days, none after thirty days, and none after three weeks. The method used was to place pigs in a small six by eight-foot pen at these different periods after it had contained an animal dead of cholera, and retain them there for one month under observation. A number of tests under varying conditions would of course be needed before any conclusions could be reached as to the length of time required for natural purification of pens and fields on the farm.

SUMMARY:

1. In natural outbreaks showing the anatomical lesions of hog cholera either bacillus cholerae suis or Th. Smith's swine plague bacteria may be present apparently acting as cause.

2. Pneumonia occurring in hog cholera is generally due to the action of swine plague bacteria.

3. Swine plague pneumonia is of frequent occurrence unassociated with contagion.

4. All the symptoms and post-mortem lesions of hog cholera of natural origin may be produced by artificial infection with pure cultures of *Bacillus cholerae suis*. This artificially induced disease is transferable by cohabitation and by inoculation from one animal to another, but does not exhibit the extremely contagious features of the natural disease.

5. Animals are easily immunized against swine plague bacteria by injection of sterilized cultures, the immunizing substance being resident in the bodies of the bacteria and not in solution in the fluid.

6. Immunity against virulent hog cholera bacilli is safely and uniformly produced by inoculation with living cultures of naturally feeble virulence, but not by sterilized cultures.

7. This double vaccination has not proved of practical value in the control of natural outbreaks in hogs.